to hGHRH(1-29)- NH_2, for: i- their increased relative binding affinity to hGHRH(1-44)- NH_2 binding sites in rat anterior pituitary *in vitro* as well as to hGHRH-R in BHK-expressing cells *in vitro*; and ii- their relative resistance to proteolysis *in vitro*.

As can be noted from Table 1 below, the relative binding affinity of the synthetic peptides with the rat GHRH receptor is not predictive of the relative binding affinity with the human receptor. As will be noted, from this point forward, GHRH analogues as presented in Table 1 will be referred to as GHRH analogues # 1 to 5.

Table 1. Priority selection based on the expected theoretical combined effects of receptor affinity and *in vitro* resistance to proteolysis on the overall bioactivity of GHRH analogues in rat anterior pituitary membrane preparations and rat serum, respectively, and of receptor affinity in BHK cell membrane preparations.

No.	Structure	Relative binding affinity in rat anterior pituitary*†	Relative binding affinity in hGHRH-R BHK-expressing cells*†	Relative resistance to proteolysis *in vitro*
1	[D-Ala^2, Ala^8, Ala^{15}, Lys^{22}] hGHRH(1-29)- NH_2	13.33 ± 0.31	499 ± 234	1.87
2	[Ala^8, Ala^9, Ala^{15}, Ala^{22}] hGHRH(1-29)- NH_2	7.74 ± 3.49	3.70 ± 0.52	1.81
3	[D-Ala^2, D-Tyr^{10}, Lys^{22}] hGHRH(1-29)- NH_2	4.90 ± 2.70	239 ± 55	2.25
4	[D-Ala^2, Ala^8, D-Tyr^{10}, Ala^{15}, D-Lys^{21}, Lys^{22}] hGHRH(1-29)- NH_2	5.00 ± 0.91	0.05 ± 0.01	6.06
5	[D-Ala^2, D-Tyr^{10}, Ala^{15}, Lys^{22}] hGHRH(1-29)- NH_2	1.04 ± 0.40	939 ± 249	3.13

GHRH analogue numbers in Table 1 correspond to numbers 13, 11, 7, 14 and 8 in Table 11 on pages 27-28 of the US patent No. 5,854,216, respectively. *, values compared to hGHRH(1-29)-NH_2; †, use of [^{125}I-Tyr^{10}]hGHRH(1-44)-NH_2 as a radioligand in structure-affinity studies.

EXAMPLE 2

Processing of the native GHRH and GHRH analogues of the present invention – Experimental assays

1- Competitive binding assay

^{125}I-GHRH binding assay was performed as previously described (Boulanger L, *et al.* (1999) Neuroendocrinology 70 : 117-127), using [^{125}I-Tyr^{10}]hGHRH(1-44)NH_2 as radioligand. Competition experiments were done in BHK (baby hamster kidney) 570

binary solvent system composed of NaClO4 0.01 M, pH 2.5 and acetonitrile. A linear gradient from 30 to 60 % acetonitrile over 45 min (rat serum) or 30 to 50% (human serum and plasma) was used. Elution of intact peptide was monitored at 214 nm and residual concentration determined by assessment of peak surface areas (Boulanger L, *et al.* (1993) Brain Res 616: 39-47; Boulanger L, *et al.* (1992) Peptides 13: 681-689).

3- *In vivo* administration of native GHRH or GHRH analogue

The ability of human GHRH analogue # 5 (human [D-Ala^2, D-Tyr^{10}, Ala^{15}, Lys^{22}] GHRH (1-29)NH_2 analogue) to stimulate GH secretion was studied in adult female rats (26-34 weeks at onset of treatment) and in a male Beagle dog.

i – *In vivo* administration into rats

Human GHRH analogue # 5 in 0.9% sodium chloride for injection USP was administered once either by intravenous (IV) or subcutaneous (SC) injection to female rats followed by a 14-day observation period, as shown in Table 2. Prior to administration, all dosing formulations were filtered using a 0.22 µm filter to ensure sterility. The actual amount of GHRH analogue # 5 administered was calculated and adjusted based on the animal's most recent body weight. Dosing started at approximately the same time each day, commencing at 9:00 am ± 30 minutes.